

REMARKS

Rejection of the claims under 35 USC 112:

Claims 8-14 have been rejected under 35 USC 112, first paragraph. The action states that the amendment contains new matter and points to the lack of a molecular weight given for succinylated PLL and pCILuc. Succinylated PLL was generated by succinylation of PLL (MW 34 kDa) thereby generating a polymer with molecular weight greater than 30 kDa. Succinylated-PLL was generated by reacting PLL with succinic anhydride (formula $C_4H_4O_3$, molecular weight 100) resulting in attachment of succinic groups to the amines on PLL (example 1, page 22-23 of the specification). pCILuc is a plasmid encoding the luciferase gene (page 26, line 20). Since the coding region for the luciferase gene is in excess of 1500 base pairs, pCILuc has a molecular weight greater than 30 kDa ($1500 \text{ base pairs} \times 0.65 \text{ kDa/base pair} = 975 \text{ kDa}$). Applicants have further shown the use of heparin (example 2), dextran sulfate (example 6) and PEGylated-SPLL (example 8) in recharging particles. Combined, these polymers represent peptide-based polyanions, modified peptide-based polyanions, sugar-base polyanions, nucleic acid-based polyanions, and PEG-modified polyanions as well as charge derived from carboxyls, sulfates and phosphates. It is the Applicants' opinion that this diverse representation of polyanions, together with the discussion of the types of polymers that can be used to recharge DNA/polycation complexes found on page 18, line 32 bridging to page 33, line 9, and on page 25, lines 11-15 of the specification provides reasonable support for the genus of all polyanion polymers having a molecular weight of at least 30 kDa.

Rejection of the claims under 35 USC 102:

Claims 8, 10, 12, 14-16 and 18 have been rejected under 35 USC 102(b) as being anticipated by Erbacher et al. Applicants have amended claim 8 to specify an ionic interaction between the polyanion polymer and polycation polymer. Erbacher et al. teach the attachment of a polyanion (BSA or streptavidin) to a DNA/polylysine complex through affinity interaction between biotin and streptavidin. The biotin is attached to the streptavidin through hydrogen bonding/van der Waals interactions between the biotin and the streptavidin. The amended claims specify ionic interaction between the polycation and the polyanion. Applicants submit Livnah et al PNAS 1993, Weber et al Science 1989, and Pugliese et al J Molecul Biol 1993 as evidence that streptavidin interaction with biotin is not ionic. Applicants have also amended

independent claim 8 to recite delivery to a cell *in vivo*. Erbacher taught only delivery to a cell *in vitro*.

Claims 8, 10, 12, 14-16 and 18 have been rejected under 35 USC 102(b) as being anticipated by Plank et al. Plank taught the formation of a complex by interacting DNA with PLL to which transferrin is covalently attached. At the time of addition of the transferrin-PLL polymer to the DNA, the transferrin was already covalently attached to the PLL. Therefore Plank et al. taught addition of a transferrin-PLL polycation polymer to DNA and not a polycation (PLL) and a polyanion (transferrin). Furthermore, as stated above, Applicants have amended claims 8 to specify an ionic interaction between the polyanion polymer and polycation polymer. Applicants have further amended the claim to specify delivery to a cell *in vivo*. Plank taught only delivery to cells *in vitro*.

Claims 8, 10, 12, 14 and 19 have been rejected under 35 USC 102(b) as being anticipated by Gao et al. Gao et al. taught the formation of a complex by interacting DNA with PLL to which transferrin (Tfn), adenovirus (AdV) or Tfn plus AdV was covalently attached. At the time of addition of the Tfn-PLL, AdV-PLL or Tfn/AdV-PLL to the DNA, the Tfn or AdV was already covalently attached to the PLL. Each of Tfn-PLL, AdV-PLL and Tfn/AdV-PLL is a polycation polymer. Therefore Gao et al. taught a complex consisting of DNA and a single polycation polymer and not DNA, a polycation polymer, and a polyanion polymer. Furthermore, as stated above, Applicants have amended claim 8 to specify an ionic interaction between the polyanion polymer and polycation polymer. Claim 19 has also been amended to specify ionic interactions between the polymers.

Claims 8, 10, 12, 14 and 19 have been rejected under 35 USC 102(b) as being anticipated by Kupfer et al. Kupfer et al. taught the formation of a complex by interacting biotin-Adenovirus and Streptavidin-polylysine with DNA and then adding transferrin-polylysine. The biotin-Adenovirus is attached to the streptavidin-polylysine through hydrogen bonding/van der Waals interactions between the biotin and the streptavidin. At the time of addition of the transferrin-PLL to the DNA, the transferrin was covalently attached to the PLL. Therefore the conjugate which was added to the DNA is a single polycation polymer and not a polycation polymer and a polyanion polymer. As stated above, Applicants have amended claim 8 and 19 to specify an ionic interaction between the polyanion polymer and polycation polymer.

Claims 8, 10, 12, 14 and 19 have been rejected under 35 USC 102(b) as being anticipated by Boussif et al. Boussif et al. taught the formation of a complex consisting of two polymers, DNA and PEI. The amended claim 8 specifies a complex consisting of a nucleic acid, a polycation, and a polyanion which is not the nucleic acid of step a). The amended claim 19 specifies a first polymer, a second polymer and a third polymer wherein the third polymer is not the nucleic acid.

Claims 8, 10, 12, 14 and 19 have been rejected under 35 USC 102(b) as being anticipated by Kabanov et al. Kabanov et al. taught the formation of a complex consisting of two polymers: DNA and a polycationic block copolymer. The amended claim 8 specifies a tertiary complex consisting of a nucleic acid, a polycation, and a polyanion which is not the nucleic acid of step a). The amended claim 19 specifies a first polymer, a second polymer, and a third polymer wherein the first and third polymers are polyanions and the third polymer is not the nucleic acid.

Claims 8, 10, 12, and 14 have been rejected under 35 USC 102(b) as being anticipated by Baker et al. Baker et al. taught the formation of a complex consisting of a bacterial artificial chromosome and either polylysine or polyethylenimine attached to adenovirus through a biotin-streptavidin affinity linkage. The Adenovirus was attached to the polycation through hydrogen bonding/van der Waals interactions between biotin and streptavidin. As stated above, Applicants have amended claim 8 to specify an ionic interaction between a polyanion polymer and a polycation polymer.

Claims 8, 10, 12, and 14 have been rejected under 35 USC 102(b) as being anticipated by Katayose et al. Katayose et al. taught the dissociation of DNA from a polycation by a highly charged polyanion. In the abstract, Katayose et al. describe the replacement of DNA in complex with PLL, resulting in release of free DNA; and not the formation of a particle consisting of DNA, PLL and poly(aspartic acid) (PAA). Katayose et al. discuss the importance of dissociation of DNA from PLL for gene delivery but offer no suggestions regarding the formation of a complex containing a polyanion other than the nucleic acid for delivery of the nucleic acid. It is the Applicants' opinion, that while Katayose et al. describe an unstable intermediate in the PAA mediated dissociation of DNA from PLL to yield free DNA, they do not teach a complex comprising nucleic acid, a polycation and a polyanion

which can be used to deliver the nucleic acid to a cell. Applicants believe that there are no statements or data in Katayose et al. which would suggest to someone practicing the art that if the intermediate could be isolated, it would be useful for delivery of a nucleic acid to a cell. The data is offered solely as evidence that DNA/PEG-PLL particle formation is not irreversible in nature, i.e. that DNA/PEG-PLL particles could be used to transfer the DNA to a cell because it is a reversible association.

Claims 8, 10, 12, 14 and 16-18 have been rejected under 35 USC 102(b) as being anticipated by Wolf et al ('067). The application states on page 12 that column 21, lines 48-55 of Wolff et al. teaches particles comprising a second polyanion. Applicants respectfully disagree. In the applicants' opinion, Wolf et al. in column 21 does not teach a second polyanion. Instead, Wolff et al. teaches the formation of a DNA/polycation complex in HEPES buffer containing the chelating agent EDTA and then crosslinking the polycation with the bifunctional crosslinking reagent DTBP. No mention is made of a second polyanion in this section. Applicants believe that the amended claims are not anticipated by binary complexes.

Rejection of the claims under 35 USC 103:

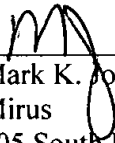
Claims 1 and 3-7 have been rejected under 35 USC 103(a) and being unpatentable over either one of Gao (1993) or Kupfer (1994) in view of Plank (1994). Gao and Kupfer each teach the attachment of a ligand, in the form of transferrin, to polylysine for the purpose of targeting DNA/PLL complexes to cells. Neither publication discusses any function of transferrin other than as a ligand to facilitate binding and endocytosis. Therefore one skilled in the art would not have been motivated by the teaching of either Gao or Kupfer to form a complex with any polyanion other than transferrin or other receptor-specific ligand. Also, as noted above in response to the 102 rejections, Gao and Kupfer each taught the addition of a transferrin-PLL cationic polymer to DNA. Neither Gao or Kupfer taught ionically associating a charged polymer to the a DNA/polycation complex in sufficient amount to form a new complex. Plank et al. taught the use of a membrane active peptide to facilitate pH-dependent endosomal disruption in cells *in vitro*. On page 12921, first column, Plank et al. taught that several polyanions did not improve delivery of DNA/polylysine complexes to cells. Specifically, Plank et al. showed that INF3, INF 2, INF4, GALA-INF1 and INF6 each had an insignificant effect on delivery of DNA to cells *in vitro*. Therefore, it is the Applicants' opinion the one skilled in the art would not have been motivated to associate a polyanion with

a nucleic acid/polycation complex to reduce the charge of the complex for delivery of nucleic acid to a cell *in vivo*.

Claims 1, 3-5, 7 and 15 have been rejected under 35 USC 103(1) as being unpatentable over Wolff et al. ('067). The action states on page 16 that Wolff et al. teach addition of polyanions to DNA/polycation complexes at column 21, lines 48-56 and at column 28, lines 1-61. The action further states while Wolff does not teach delivery of the complexes to cells, it would have been obvious to deliver the complexes because Wolff teaches the complexes are intended to be used to transfer nucleic acids to cells *in vivo*. It is the applicants opinion that Wolff et al '067 only provides for delivery of caged or uncaged DNA/polycation complexes to cells (see column 13, lines 44-49, examples 1-10, and Figs. 1, 4, 5 and 6). It is also the applicants' opinion that the teaching of Wolff et al., at column 28, is that caged DNA/polycation complexes are more stable than uncaged DNA/polycation complexes. Polyanions are utilized by Wolff et al. to compete with DNA interaction with polycations. This competition provided a means to measure stability of caged vs. uncaged DNA/polycation complexes. This interpretation is supported by lines 62-67 of column 28. Wolff et al. also teach the use of polyanions to disrupt DNA/polycation complexes at column 24 lines 51-62. As stated above in response to the 112 rejections, applicants find no mention of polyanions at column 21 lines 48-56.

The Examiner's objections and rejections are now believed to be overcome by this response to the Office Action. In view of Applicants' amendment and arguments, it is submitted that claims 1-4, 6-9, 11-14, 16-22, 24-26, 28-31, 33-36 and 39-40 should be allowable and Applicants respectfully requests an early notice to such effect.

Respectfully submitted,


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I hereby certify that this correspondence is being sent
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